Comparison of Chlamydia infection prevalence between patients with and without ectopic pregnancy using the PCR method

Porównanie częstości występowania zakażeń Chlamydia u pacjentek z ciążą ektopową i bez, przy użyciu metody PCR

Naderi Tayebeh[1], Kazerani Fatemeh[2], Bahrampoor Abbas[3]

[1] Department of Obstetrics and Gynecology, Kerman University of Medical sciences and Health services, Iran
[2] Obstetrics and Gynecology , Rafsanjan University of Medical sciences and Health services, Iran
[3] Biostatistics, Kerman University of Medical sciences and Health services, Iran

Abstract

Objectives: *Damage of the fallopian tube after sexually transmitted diseases like Chlamydia trachomatis, is an important risk factor for ectopic pregnancy (EP). The study was designed to assess the prevalence of C. trachomatis infection in patients with EP in the southeastern part of Iran.*

Method: *The polymerase chain reaction (PCR) on fallopian tube tissue was applied to detect Chlamydia DNA in 42 patients with EP (EP group) and 87 patients without EP (control group) who underwent tubal ligation. The same protocol was performed with urine samples taken from the husbands in both groups.*

Results: *Out of all studied females, 5 patients in the EP group were PCR-positive for C. trachomatis and none of the control group subjects was PCR-positive for C. trachomatis infection ($P < 0.05$). Among the husbands, the PCR result was positive in the urine of 19 males (9 in the EP group and 10 in the control group). All PCR-positive women had husbands with PCR positive urine samples. No significant difference was found between Chlamydia infection in the EP and the control groups regarding age, duration of marriage, contraceptive method and history of infertility, surgery and pelvic pain. There was no significant difference between prevalence of EP in women based on the PCR outcome in the husbands. The Chlamydia infection in men did not show any relation to the number of marriages.*

Conclusion: *Based on our findings, it can be concluded that Chlamydia is an important risk factor of the fallopian tube damage and EP in our society. Therefore, screening programs and treatment of Chlamydia infection are recommended in young women and high risk women and men.*

Key words: **ectopic pregnancy** / **chlamydia** / **prevalence** /

Corresponding author:
Tayebeh Naderi
Department of Obstetrics and Gynecology, Kerman University of Medical sciences and Health services, Iran
P.O. Box: 7616914111, Kerman, Iran
tel: +983413222250; fax: 983413222763
e-mail: tayebeh_ndr39@yahoo.com; Swt_f@yahoo.com

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Streszczenie

Cel pracy: *Cel pracy: Uszkodzenie jajowodu po przebyciu choroby przenoszonej drogą płciową, takiej jak infekcja Chlamydia trachomatis jest istotnym czynnikiem ryzyka ciąży ektopowej (EP). Celem pracy była ocena częstości występowania zakażenia C. trachomatis u pacjentek z ciążą ektopową w południowo-wschodniej części Iranu.*

Materiał i metoda: *Reakcję łańcuchowej polimerazy (PCR) w celu wykrycia DNA Chlamydii przeprowadzono na tkance jajowodu pobranego od 42 pacjentek z ciążą ektopową (grupa EP) i od 87 pacjentek bez ciąży ektopowej (grupa kontrolna) w trakcie podwiązania jajowodów. Ten sam protokół przeprowadzono z próbkami moczu pobranymi od mężów pacjentek obu grup.*

Wyniki: *Z całej grupy badanej, 5 pacjentek z ciążą ektopową miało pozytywny wynik badania PCR na obecność C.trachomatis, żadna pacjentka z grupy kontrolnej nie miała pozytywnego wyniku (p<0,05). Badania moczu mężów wykazały dodatni wynik PCR u 19 mężczyzn (9 w grupie z EP i 10 w grupie kontrolnej). Wszystkie kobiety z dodatnim wynikiem PCR miało mężów również z dodatnim wynikiem PCR z moczu. Nie znaleziono istotnych różnic pomiędzy infekcją Chlamydia w grupie z EP i grupie kontrolnej w odniesieniu do wieku, czasu trwania małżeństwa, metody antykoncepcyjnej i wywiadem w kierunku niepłodności, przebytych operacji i bólu w miednicy mniejszej. Nie znaleziono istotnej różnicy pomiędzy częstością występowania ciąży ektopowej u kobiet w oparciu o wynik badania PCR u męża. Infekcja Chlamydia u mężczyzn nie była związana z ilością małżeństw.*

Wnioski: *Na podstawie naszych badań, można stwierdzić, że Chlamydia jest ważnym czynnikiem ryzyka uszkodzenia jajowodu i wystąpienia ciąży ektopowej w naszym społeczeństwie. Dlatego program badań przesiewowych i leczenia zakażenia Chlamydia są zalecane u młodych kobiet oraz u kobiet i mężczyzn z grupy wysokiego ryzyka.*

Słowa kluczowe: **ciąża ektopowa** / **chlamydia** / **częstość** /

Introduction

Ectopic pregnancy (EP) is implantation of a fertilized egg outside the endometrial cavity. The rate of EP is rising due to an increase in sexually transmitted diseases (STDs), ovulation induction, use of assisted reproductive technologies (ART), tubal ligation and high accuracy of the available diagnostic methods. Fallopian tube is the most common site of EP. Any factor which interferes with the natural mechanism of tubal transfer of the fertilized egg can be a predisposing factor for EP. In most women there are one or more recognized risk factors for EP. In women with proved tubal pathology the risk increases at least 2 times [1].

Age is the main predisposing factor for EP, followed by having frequent partners, new partner, infected partner, temporary use of barrier methods, and use of oral contraceptives (OCP) [2]. Chlamydia trachomatis has been reported to be the most common bacterial pathogen of STDs and the main cause of urethritis, cervicitis, PID and an important cause of EP, infertility and chronic pelvic pain [3, 4].

Tubal pregnancy has been reported to be three times more common in women who had anti-chlamydia antibody titration more than 1.64 [5]. In Gerard et al. study, 7 among 10 specimens of fallopian tubes in patients with EP were positive for chlamydia DNA detected by PCR [6]. In a study by Barlow et al., PCR and in-situ hybridization (ISH) were applied to detect chlamydia DNA in fresh specimens (endometrium, fallopian tubes and ovary) which was positive in 56% and 67% of patients with EP and 71% of infertile women [7].

Previous infection with chlamydia is associated with an increased risk of EP which is significant in young women [8]. According to the study by Menon et al., prevalence of EP was lower in women under 20 years of age in comparison to those over 20 [9].

In most cases, tubal damage occurs due to STDs, among which gonorrhea and chlamydial infections are the most prevalent infectious causes. About 70-80% of women and a high percentage of men with Chlamydia infection are asymptomatic [10].

The prevalence of chlamydia infection based on the population has been reported to be 1-40%. There is evidence regarding racial differences in prevalence of Chlamydia infections [8]. Since there is limited evidence about current prevalence of Chlamydia infection in patients with EP in Iran, the authors of this study decided to determine the rate of this condition.

The main goal of this study was to investigate the rate of C. trachomatis infection in patients with EP in the southeastern part of Iran.

Materials and methods

The study was conducted on 42 women with EP who were candidates for laparotomy and salpingectomy and 87 others without EP who were candidates for TL in Afzalipour Hospital, Kerman, Iran. Written informed consent was obtained from the participants prior to the study and data were gathered using a structural questionnaire.

During the surgery a specimen from the fallopian tube was taken and sent to the laboratory. A specimen of first void urine was taken from the husbands in both groups. These specimens were frozen at -70°C. Following gathering the required specimens, DNA was extracted from both collected specimens (fallopian tube and urine) using DNA extraction kit. Specific primers of Cymatia Trachomatis were applied for PCR. After PCR, electrophoresis was performed in agarose jell 1.5% with positive and negative controls. Existence of 325 bp bound in the series was considered to be a positive result.

The study was approved at Kerman University of Medical Sciences Ethics Committee.

Statistical analysis

Results were reported as mean ± standard deviation (SD) or median for quantitative variables and percentages for categorical

variables. The groups were compared using the Student's t- test for continuous variables and the chi-square test (or Fisher's exact test if required) for categorical variables. P values of 0.05 or less were considered statistically significant. All the statistical analyses were performed using SPSS version 13 (SPSS Inc, Chicago, IL, USA) for Windows.

Results

The fallopian tube specimens PCR results were positive for C. *trachomatis* infection in 5 patients (3.88%) and negative in the remaining 124 females (96.1%). The five patients whose fallopian tube samples were positive for Chlamydia infection all belonged to the EP group. None of the 87 women without EP had a positive result ($P < 0.05$).

No significant difference was seen in terms of mean (±SD) age of women with positive PCR (31.2±6.57 years) and negative PCR (31.6±5.64 years). Similarly, mean duration of marriage in groups with negative and positive results was not found to be significantly different (6.8 years vs. 10.63 years, $P > 0.05$).

Among 5 women with positive PCR, 4 used withdrawal method and 1 patient consumed OCP as contraceptive method. There was no significant association between the PCR result and the method of contraception.

The PCR outcome of urine specimens was negative in 110 persons (86.05%) while it was positive in 19 ones (13.95%). Among males with the negative result, none of their wives had a positive PCR result, while among 19 ones with positive results, in 5 cases their wives also had positive results. However, a significant difference for prevalence of infection in women with and without EP based on the infection of the husbands ($P > 0.05$) was not confirmed.

One of the males with the positive result had been engaged two times; no statistical difference was found between the PCR result and number of marriage. Among 42 women with EP, in 9 cases the husband had positive result while in 87 women without EP; in 10 cases the husband was PCR-positive. There was no significant difference for prevalence of EP based on the PCR results of infection in males.

Discussion

Since among STDs, C. *trachomatis* is one of the prevalent agents responsible for damage to the fallopian tube and subsequent higher risk for EP, this study was designed to assess the role of chlamydia infection in tubal damage and EP among women in our society.

In this study no significant association was found between factors previously mentioned as predisposing for chlamydia infection, namely age and duration of marriage [8]. There was only one person with more than on marriage in our study. However, if we intend to assess the role of frequent marriages in chlamydia infection, which is mentioned in other studies [11], we should conduct a study about EP rate in multi-partners or people with a new partner or an infected one. There was no significant relation between pelvic pain, infertility and PCR results; in order to investigate these issues, maybe a larger sample size will be more suitable.

Frequent use of barrier methods, consumption of OCP and IUD have been mentioned as risk factors in various references [11].

In our study, 4 patients among those who used withdrawal method and 1 woman with OCP were PCR positive for Chlamydia infection, however, the difference was not significant. No cases of infection were detected among those who used other methods of contraception. Prevalence of infection was higher among women who did not use specific methods such as barrier and hormone.

There was a significant difference about prevalence of infection detected by PCR between women and men which can be a considerable point in the screening program of men, since Chlamydia infection is the cause of 30-40% of urethritis and 50% of epididymitis [12] among the males and can cause prostatitis, urethral stenosis and probably infertility [13].

Among the total number of the 19 males with the PCR positive results, 9 were in the case group and the remaining 10 were for control group; however a significant association was not confirmed between the prevalence of EP and PCR results among males, but the number of cases with positive PCR was about 2 times in cases compared to controls. All the five women with positive PCR were in the case group and the difference between the two groups was significant.

Thus, based on our findings C. *trachomatis* could be mentioned as one of major risk factors in STDs and subsequent tubal damage and increasing rate of EP in our society. Screening program and treatment of Chlamydial infection is recommended in young women and high risk males and females.

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